STEROID SAPONINS OF *Trillium kamtschatcense*

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We have previously [1] reported the isolation from the roots of Trillium kamtschatcense of two glycosides which we called trillosides A and B. In the present paper we describe the results of the determination of the structures of these compounds.

A methanolic extract of the roots, after demineralization on Sephadex G-25, was separated by column chromatography on silica gel into two fractions: trillosides A and B in a ratio of 3 : 1. Acid hydrolysis of the saponins and subsequent chromatography showed that the genin in trillosides A and B is diosgenin. Its structure was also confirmed by a comparison of analytical and spectral (IR, PMR, and mass spectrometry) results with those given in the literature [2-7]. Paper chromatography (PC) showed the presence in a hydrolyzate of trilloside A of glucose and rhamnose and in a hydrolyzate of trilloside B of only glucose. The molecular weights of the saponins determined from the yield of genin were 896 for trilloside A and 920 for trilloside B. Consequently, trillosides A and B are triosides of diosgenin.

To determine the types of bonds between the monosaccharides in trillosides A and B we used the method of exhaustive methylation [8]. After methanolysis of permethylated trilloside A its hydrolyzate was shown by gas–liquid chromatography (GLC) to contain methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucopyranoside and methyl 2,3,4-tri-O-methyl-α,β-L-rhamnopyranoside, and also methyl 3,6-di-O-methyl-α,β-D-glucopyranoside in a ratio of 1 : 1 : 1. The nature of the permethylated monosaccharides from a methanolyzate of trilloside A was also confirmed by mass spectrometry [9].

For a more reliable determination of the structure of the di-O-methyl ether of glucose isolated from the methanolyzate of trilloside A, it was methylated to completion with trideuteromethyl iodide. The mass spectrum of the compound obtained corresponded to that calculated theoretically for a methyl 3,6-di-O-methyl-2,4-di-O-trideuteromethylhexose [10].

Thus, trilloside A is a trioside of diosgenin. The sugar directly attached to the genin is glucose, and residues of glucose and rhamnose are attached to this in positions 2 and 4. To determine the positions of their bonds with the first glucose residue in trilloside A, this saponin was subjected to acetolysis. This gave several compounds, a scheme of the formation of which is shown on the following page.

The structure of the diosgenin monoglucoside (II) was established in the following way: after acid hydrolysis, glucose and diosgenin were identified by chromatography on paper and on silica gel, while in the products of the methanolysis of (II) GLC showed the presence of only methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucopyranoside. Finally, the structure of (II) can be confirmed by a study of its mass spectrum, which is given on the following page.

As can be seen, the peak of the molecular ion of compound (II) (m/e 632) has a low intensity. However, its presence unambiguously shows that compound (II) is a diosgenin monohexoside. Furthermore, the presence of peaks with m/e 219, 187, 155, 101, and 88, and some others, and the results of chromatography, given above, reliably characterize the sugar residue in the molecule of the monohexoside (II) as glucose.

The spectrum, which is given below, also reliably confirms the structure of the aglycone. As is well known [3] (see Experimental), the formation of an ion with m/e 139 and 115 showing the presence of a

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Acetolysis of Trilloside A

I

1. Ac_2O/H_2SO_4 Division
2. CH_3ONa/CH_3OH
3. CH_3I/NaH

II + III + IV

spirostan ring in it is the most characteristic for free diosgenin. In the spectrum of compound (II) the peaks with m/e 115 and 139 are among the strongest. In addition to this, there are other peaks (m/e 254, 267, 271, 282, etc.) characterizing the aglycone as diosgenin.

The strongest peak in the spectrum of compound (II) is that of an ion with m/e 397, corresponding to the ejection of a sugar residue from the molecular ion. The spectrum of compound (II) also contains a number of other ions with m/e greater than the molecular weight of the genin. However, they are of low

m/e	Intensity, %	m/e	Intensity, %	m/e	Intensity, %
43	22	125	12	253	30
45	48	127	13	254	7
55	25	131	12	267	4
69	41	133	16	271	14
71	19	135	10	282	28
73	17	139	53	283	31
75	21	143	14	284	6
79	12	145	19	299	4
81	23	147	26	324	6
83	12	149	20	325	6
85	10	155	10	327	5
88	78	157	14	328	5
89	22	158	8	379	7
91	12	161	19	396	50
93	17	171	6	397	100
95	19	173	8	398	44
97	14	175	14	426	4
99	13	181	6	427	5
101	80	159	17	380	2
102	10	187	30	428	3
105	17	197	3	440	4
107	17	199	5	441	7
109	13	203	6	422	2
111	23	204	5	453	3
115	24	205	5	456	1
117	8	219	8	466	4
119	14	235	3	467	2
121	22	249	6	632	2

intensity and their formation is apparently due to specific cleavages both of the monosaccharide residue and of the genin molecule.

Thus, the facts presented on the hydrolysis of the unmethylated (II), the methanolysis of (II), and the mass spectrum reliably confirm its structure as a diosgenin monoglucoside.

The results of the methanolysis of compound (III) and of a subsequent study of the products of its decomposition by GLC have shown that they contain fully methylated glucose and 2,3,6-tri-O-methyl-D-glucose in a ratio of 1 : 1. This shows that in the molecule of trilloside A the terminal glucose occupies position 4 of the glucose residue attached directly to the genin. Consequently, the rhamnose residue must be attached to C_2 of this glucose residue. This is also shown by the fact that in the products of the methanolysis of compound (IV) completely methylated rhamnose and 3,4,6-tri-O-methyl-D-glucose (1 : 1) were found by GLC. Consequently, the structure of trilloside A can be represented by structure (I).

Trilloside B has a similar structure. In a methanolyzate of permethylated trilloside B, methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucopyranoside (its structure was also confirmed by mass spectrometry) and methyl 3,6-di-O-methyl-α,β-D-glucopyranoside (2 : 1) were identified by GLC. This shows that trilloside B is a diosgenin trioside and the glucose residue attached directly to the genin bears side chains at C_2 and C_4 which also consist of D-glucose.

EXPERIMENTAL

Chromatography was performed with type KSK silica gel and paper of type "M" ["slow"] of the Volodarskii Leningrad mill. The saponins and genins were revealed with Sannié's reagent [1] or by spraying with sulfuric acid and subsequent heating of the plates, and the free sugars were revealed with aniline phthalate.

The IR spectra of the substances were recorded on a UR-10 instrument (KBr tablets), the PMR spectrum of diosgenin on a Varian A-60 spectrometer (chloroform), and the mass spectra on a Varian CH-6 instrument with an ionization potential of 70 eV and a temperature of the evaporator of 180°C. Gas–liquid chromatography was performed on an LKhM-8MD instrument (column containing 5% of PNPGS on Chromosorb W, 80-100 mesh). Melting points were determined on a Kofler block. The analyses of all the compounds corresponded to the calculated figures.

Isolation of the Saponins. The air-dry roots of *Trillium kamtschatcense* (1 kg) were extracted successively in a Soxhlet apparatus with chloroform and methanol. The methanolic extract was evaporated at 40-50°C, and the residue was dissolved in water and extracted with ether. The extracted aqueous solution was evaporated and the residue was dissolved in the minimum amount of water and transferred to a column of Sephadex G-25. Elution was performed with water and the fractions giving a positive reaction for saponins were collected. The yield of a mixture of trillosides A and B was 16 g (1.6%).

Separation of Trillosides A and B. The combined trillosides A and B (3 g) were transferred to a column of silica gel and eluted in the butan-1-ol–acetic acid–water–ethyl acetate (6 : 4 : 3 : 2) system, 15-ml fractions being collected. The separation was monitored by TLC in the same system. The yield of trilloside A was 1.9 g, mp 196-200°C, $[\alpha]_D^{26}$ − 2.2° (c 1.3; methanol) and that of trilloside B 0.6 g, mp 182-186°C, $[\alpha]_D^{26}$ + 0.5 (c 1.1; methanol).

Acetylation of Trillosides A and B. Trilloside A (0.15 g) was dissolved in a mixture of 3 ml of absolute pyridine and 1 ml of acetic anhydride. The mixture was kept at room temperature for a day. After treatment with water, the precipitate was collected and was purified by preparative TLC on silica gel, mp 124-127°C (methanol), $[\alpha]_D^{26}$ − 56° (c 2.4; chloroform), $C_{45}H_{72}O_{17}$. The acetate of trilloside B was obtained similarly, mp 134-137°C (methanol), $[\alpha]_D^{26}$ −34° (c 2.0; chloroform), $C_{45}H_{72}O_{18}$.

Identification of the Sugars in Trillosides A and B. Trillosides A and B (0.02 g each) were hydrolyzed separately in 4% hydrochloric acid at 100°C for 5 h. The mixture was cooled, the precipitate was filtered off, and the filtrate was neutralized with Amberlite IRA-410 anion-exchange resin (HCO_3^-), and paper chromatography in the butan-1-ol–pyridine–water (6 : 4 : 3) system showed the presence of glucose and rhamnose in the hydrolyzate from trilloside A and of glucose alone in that from trilloside B.

Identification of the Aglycone of Trillosides A and B. The combined saponins (6 g) were heated with 70 ml of 5% hydrochloric acid at 100°C for 6 h. Then the mixture was cooled, and the precipitate was filtered off, washed on the filter with water, and dried. It was recrystallized from acetone. Yield 0.5 g, mp 206-208°C, $[\alpha]_D^{26}$ − 121° (c 2.1; chloroform), $C_{27}H_{42}O_3$. Literature data for diosgenin: mp 204-205°C,

$[\alpha]_D^{32}$ −122.5° (c 0.8; chloroform) [7]. The mass spectrum of the compound isolated contained the following peaks, with m/e: 43 (32%), 53 (40%), 69 (53%), 115 (44%), 139 (100%), 253 (19%), 267 (34%), 271 (45%), 282 (87%), 300 (54%), 342 (31%), 345 (9%), 355 (12%), 396 (2%); 399 (2%), and 414 (29%); and its acetate 43 (28%), 56 (17%), 69 (15%), 81 (15%), 107 (13%), 115 (10%), 121 (15%), 139 (25%), 158 (15%), 161 (12%), 253 (17%), 267 (15%), 282 (100%), 283 (25%), 299 (2%), 303 (3%), 313 (13%), 315 (7%), 327 (2%), 375 (2%), 396 (71%), 397 (23%), and 456 (0.8%). The peaks listed and the ratio of the intensities agree well with those given in the literature [3, 4].

In the PMR spectrum of this compound, the protons of the methyl groups at C_{18} and C_{27} have chemical shifts of δ = 0.79 (6H) and those at C_{19} and C_{21} shifts of δ = 0.97-1.05 (6H), and the $C_{16}\alpha$ and C_{26} protons have shifts of 4.40 ppm (1H) and 3.4 ppm (1H), respectively (see [2]). The IR spectrum of the compound was identical with that of diosgenin [5].

Determination of the Types of Bonds between the Monosaccharides in Trillosides A and B. Trilloside A (1 g) and trilloside B (0.3 g) were methylated by Hakomori's method [8]. After purification of the products by column chromatography on silica gel, 0.8 g of permethylated trilloside A was obtained with $[\alpha]_D^{26}$ −43° (c 1.9; chloroform) and 0.1 g of permethylated trilloside B.

The permethylated trilloside A (0.5 g) was dissolved in 20 ml of a 1 N solution of hydrochloric acid in absolute methanol, and the solution was heated at 100°C for 5 h. The reaction mixture was diluted with water, the precipitate was filtered off, the filtrate was neutralized with Amberlite IRA-410 anion-exchange resin (HCO_3^-) and evaporated at 30°C. Part of the product was separated by column chromatography on silica gel, giving methyl 2,3,4,6-tetra-O-methyl-D-glucopyranose, methyl 2,3,4-tri-O-methyl-D-rhamnopyranose, and methyl 3,6-di-O-methyl-D-glucose. The permethylated monosaccharides were identified by GLC and mass spectrometrically [9], and the 3,6-di-O-methylglucose by GLC, the mass spectrum of its trideuteromethylated derivative agreeing well with that calculated theoretically [10] and containing the following peaks, with m/e: 45 (23%), 48 (13%), 54 (12%), 55 (13%), 71 (8%), 73 (8%), 74 (13%), 75 (61%), 76 (11%), 78 (7%), 81 (8%), 104 (33%), 107 (40%), 120 (13%), 128 (10%), 130 (20%), 133 (10%), 149 (20%), 150 (2%), 151 (2.1%), 182 (8%), 193 (8%), 225 (0.6%), 256 (0.4%).

By the GLC method, the ratio of the permethylated glucose and rhamnose and the 3,6-di-O-methyl-D-glucose in the methanolyzate of trilloside A was found to be 1 : 1 : 1. Similarly, for trilloside B the ratio of permethylated glucose and its 3,6-di-O-methyl ether was found to be 2 : 1.

Acetolysis of Trilloside A. Trilloside A (1 g) was dissolved in 20 ml of acetic acid−acetic anhydride−concentrated sulfuric acid (10 : 10 : 0.25) and the solution was stirred at room temperature for 30 min. Then it was treated with sodium bicarbonate and was evaporated, and the residue was acetylated with acetic anhydride in pyridine. The acetylation products were extracted with chloroform and after the elimination of the acyl protection with sodium methoxide in methanol they were separated on a column of silica gel in the butan-1-ol−acetic acid−water−ethyl acetate (6 : 4 : 3 : 2) system. The separation and subsequent methylation gave the products shown in the scheme.

A. Determination of the structure of the monoglucoside (II). Glucose was identified by paper chromatography in the products of the hydrolysis of the monoside (II) with 4% hydrochloric acid in 50% ethanol. Diosgenin was identified by TLC on silica gel together with an authentic sample. After the methanolysis of (II), methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucopyranoside was identified by GLC.

B. Determination of the structures of the biosides (III) and (IV). Methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucoside and methyl 2,3,6-tri-O-methyl-α,β-D-glucoside in a ratio of 1 : 1 were found in the products of the methanolysis of the permethylated bioside (III) and methyl 2,3,4-tri-O-methyl-α,β-L-rhamnoside and methyl 3,4,6-tri-O-methyl-α,β-D-glucoside in a ratio of 1 : 1 in the corresponding products from (IV).

SUMMARY

Glycosides − trillosides A and B − have been isolated from the roots of Trillium kamtschatcense.

The structure of trilloside A has been established as 4-O-(D-glucopyranosyl)-2-O-(L-rhamnopyranosyl)-D-glucopyranosyl-(1 → 3)-diosgenin. Trilloside B is 2,4-di-O-(D-glucopyranosyl)-D-glucopyranosyl-(1 → 3)-diosgenin.

LITERATURE CITED

1. V. P. Konyukhov, A. F. Sviridov, and B. S. Subbotin, Collection of Papers from the Khabarov Pedagogic Institute [in Russian] (1971).
2. D. H. Williams and N. S. Bhacca, Tetrahedron, 21, 1641 (1965).
3. H. Budzikiewicz, J. M. Wilson, and C. Djerassi, Monatsh., 93, 1033 (1962).
4. H. J. M. Fitches, Advan. Mass Spectrometry, 2, 428 (1963).
5. R. N. Jones and F. Herling, J. Org. Chem., 19, 1252 (1954).
6. R. N. Jones and A. R. H. Cole, J. Amer. Chem. Soc., 74, 5648 (1952).
7. G. B. Iskanderov, Khim. Prirodn. Soedin., 633 (1970).
8. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
9. N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, and B. M. Solotarev, Tetrahedron, 19, 2209 (1963).
10. N. K. Kochetkov and O. S. Chizhov, Tetrahedron, 21, 2029 (1965).
11. C. Sannié and H. Lapin, Bull. Soc. Chim. France, 11-12, 1080 (1952).